BIOCHEMISTRY STUDIES IN BLOOD AND BODY FLUIDS OF TUBERCULAR PATIENTS

Dr. Syed Shabbar Masih

Assistant Professor Dept. of Biochemistry Venkateshwara Institute of Medical Sciences National Highway-24, Rajabpur, Gajraula, Distt. Amroha (U.P.)- 244236

ABSTRACT:

Background: One of the oldest diseases known to impact humans, tuberculosis is a leading cause of death in the entire world. The lungs are typically affected by this condition, which is brought on by bacteria from the Mycobacterium TB complex, though other organs may also be involved in up to one-third of cases. TB caused by drug-susceptible strains is almost always curable if properly treated. In 50–65% of instances, the condition may be deadly if left untreated within 5 years. Typically, infected pulmonary TB patients create droplet nuclei that are disseminated through the air by other patients. According to the WHO study "TB in the South-east Asia Area," India reported 1.9 million new cases in 2006. 9.2 million new TB cases were reported globally during this time. HIV infection affected 1.2 percent of all new cases in the nation, and multi-drug resistant TB affected 2.8 percent of all new cases (MOR-TB). A strong index of suspicion is essential for the diagnosis of tuberculosis.

Aim: The aim of the present study is Biochemistry studies in blood and body fluids of tubercular patients. Evaluation and significance of ADA activity and CRP in blood and body fluids of tubercular patients.

Material and Method: The present study "Biochemical Studies in Blood and Body Fluids of Tubercular Patients" was carried out in Department of Biochemistry. The cases under study included pulmonary and other sites tuberculosis, non-tubercular disease cases and age &sex matched healthy controls. A total of 50 real cases of pulmonary tuberculosis were chosen for the study in group B, along with 50 instances of unrelated illnesses in group C and 50 healthy, age- and sex-matched controls in group A. The controls and study group's blood was drawn via venipuncture, transferred to an EDTA vacutainer, utilised for haematological testing, and serum was used for the examination of ADA, CRP, and IFN-γ.

Results: Conventional markers of TB i.e., serum ADA and serum CRP levels are significantly higher in tuberculous patients as compared with the healthy age & sex matched controls and other non-tubercular disease cases. The levels of new markers, serum IFN-γ found to be significantly higher in TB patients compared to non-tubercular cases and healthy control included in the study. The levels of serum ADA and serum CRP were also found to be higher in various body fluids in those suffering with tuberculosis compared to that with non-tubercular diseases associated fluid accumulation. It is also concluded that there is significant positive co-relation between bacterial positivity and body fluid ADA and CRP levels. Similarly, there is also significant positive co-relation observed between serum ADA and serum CRP levels between tubercular and non- tubercular cases

Conclusion: Based on the findings of the current study, it can be deduced that iron deficiency anaemia is linked to the production of free radicals, anomalies, and peroxidation of essential body molecules, increasing the danger for both pregnant women and the developing baby. To evaluate the status of

antioxidant in pregnancy-related disorder, more extensive research is required. During a diabetic pregnancy, gestational diabetes causes an oxidative stress situation that makes membrane damage and lipoperoxidability easier to occur.

INTRODUCTION

One of the oldest diseases known to impact humans, tuberculosis is a leading cause of death in the entire world. Although other organs may be involved in up to one-third of cases, the lungs are typically affected by this disease, which is brought on by bacteria from the Mycobacterium TB complex. One of the terrible diseases that many people in underdeveloped nations, including India, suffer from is tuberculosis (TB). One of the most widespread illnesses, it has a significant mortality and morbidity rate. The total number of infections is increasing every year as a result of the country's rapidly expanding population, sociocultural factors, the rise in HIV cases, the number of the poor, and non-compliance with treatment. Public health professionals are concerned about the broad development of TB cases that are resistant to various drugs because it not only makes the illness condition more dangerous but also necessitates very expensive treatment. [1]

TB has long been an endemic illness throughout the nations of the Indian subcontinent. 95% of TB cases, which affect one third of the global population, occur in underdeveloped nations.[2] The majority of the population is young, which has a significant impact on productivity in less developed nations. The severity of TB's impact in poorer nations raises serious public health issues. Such a great burden of infectious diseases has great economic burden on the financial condition of the country.

In underdeveloped nations, TB is still one of the leading causes of mortality and morbidity. Although the lung is the organ that is most frequently affected, inflammation of serous membranes is also common.[3] When typical histological characteristics can be shown, or when mycobacteria can be isolated from bodily fluids, sputum, or stomach lavage, the

conclusive diagnosis is made. There are also a number of additional techniques, including gel electrophoresis, radiometric assays, and polymerase chain reactions.[4] The difficulty and length of time required for mycobacteria isolation and culture, as well as the complexity and technical difficulty of other tests, are well known. The majority of the time, a diagnosis is made based on radiological characteristics, clinical characteristics, and positive tuberculin testing.[5] Recently, efforts have been made to identify the most straightforward methods that are equally effective as well as dependable, more practical, affordable, and quick to produce results.

Timely diagnosis of TB remains challenging for clinical laboratories. IGRAs have a potential to serve as a useful test to confirm TB when microbiological diagnosis is hard to establish, IGRAs are useful in excluding active diseases, they must be highly sensitive in patients with TB. The TB testing using IFN-v is now been successfully implemented, no adverse programmatic outcomes were identified. Surveillance for TB now appears possible. Program considering switching from TST to IFN-v may expect to realize benefits in completion test for TB testing reporting and surveillance capacity. Thus, CRP estimate is a quick test to help in TB diagnosis. It also depicts the progression of the illness and the efficacy of treatment.[6]

Tillett and Francis first identified CRP (C-reactive protein) in the serum of individuals with acute inflammation in 1930 as a molecule that interacted with pneumococcus C-polysaccharides. It was once assumed to be a pathogenic secretion due to the fact that it is raised in a number of illnesses, including carcinomas. That argument was settled by the discovery of CRP synthesis and secretion in the

liver. The goal of the current study was to determine the value of ADA, CRP, and IFN-v levels in blood and other body fluids for the diagnosis of tuberculosis. To determine the diagnostic and prognostic value, it was also necessary to examine the interactions between all of these characteristics and their co-relation and comparison.[7]

The study was undertaken to estimate the biochemical parameters like ADA, CRP and IFN-v in blood as well as various body fluids that are tapped from tuberculous patients. These biochemical parameters were studied in tuberculous patients and were compared to normal healthy subjects and with another group of patients with non-tuberculous disease. [8]

MATERIAL AND METHODS

The present study "Biochemical Studies in Blood and Body Fluids of Tubercular Patients" was carried out in Department of Biochemistry. The cases under study included pulmonary and other sites tuberculosis, non-tubercular disease cases and age &sex matched healthy controls.

Sr. No.	Study Groups	Sample Size
Group A	Control Healthy Subjects	50
Group B	Cases (TB)	50
Group C	Cases (Diseases other than TB)	50

Specimen collection:
A) Blood samples of the controls/study group was collected for various biochemical analyses by venipuncture and transferred to EDTA vacutainer and used for hematological tests and serum was used for analysis of ADA, CRP and IFN-y.
B) Body fluids like pleural, peritoneal, and pericardia! and CSF fluids all were collected in sterile tubes.

A) **Hematological Tests:** (Dacie and Lewis 2006)[9]
1) Total white blood cell count was done by automated cell counter. Normal Range 4.0-10.0 x 109 /μ
2) Differential leukocyte count was done by stain blood film
3) ESR by Wintrobe's method
4) Ziehl Neelsen's (Z-N) staining for AFB: In case of all sputum, fluids will be subjected to staining for AFB by Z-N technique

Biochemical Tests:
1) Measurement of ADA activity (Giusti and Galanti 1974) [10]
2) Estimation of C - reactive protein (Kidmark 1972) [11]
3) IFN, by QFTIFN - v assay method (Mori et al. 2005) [12]
4) Analysis of ADA in body fluids (Pleural, pericardia, CSF and peritoneal)
5) Estimation of CRP in body fluids (Pleural, pericardia, CSF and peritoneal)

Statistical analyses: First the arithmetic mean (X), standard deviation (S.D.) & standard error of mean (SEM) Pearson's Correlation Coefficient (r) were calculated by using the formula; Graph Pad Prism and Microsoft Office Excel were used for statistics and graph.

RESULT: -
The results that were calculated from the data collected are mentioned in tabular format as follows. Total 50 authentic pulmonary TB patients were chosen for study in group B, while group C consisting of 50 cases of non-tubercular diseases with and group A consisting of 50 normal healthy age and sex matched controls.

Table 1: Serum CRP, IFN-α, ADA, levels in group A, B and C (mean ± S.D.).

	Group A (n=50)	Group B (n=50)	Group C (n=50)
CRP (mg/dl)	0.35±0.03	1.00±0.29	0.60±0.11
IFN-γ (IU/L)	0.36±0.10	0.88±0.14	0.40±0.12
ADA (U/L)	8.10±0.29	12.22±1.10	7.33±0.82

The serum CRP levels were estimated and compared among all the three groups and a significant difference was found in the study groups.

Table 2: Comparison of ADA levels in body fluids of group B and C subjects (mean ±S.D.).

ADA (U/L)	Group B (n=50)	Group C (n=50)
Pleural	19.51±2.83	9.22±1 .11
Pericardial	18.43±0.73 .01	7.08±0.44
Peritoneal	23.81±1.13	9.36±1.00
CSF	24.30±1 .55	9.02±0.66

ADA estimation was done from the fluids of subjects in group B and Group C and it was found that there was statistically significant difference between the ADA levels of body fluids in group B and group C subjects.

Table 3: Comparison of CRP levels in body fluids of Group B and C subjects (mean ± S.D.).

CRP (mg/dl)	Group B (n=50)	Group C (n=50)
Pleural	1.03±0.02	0.50±0.01
Pericardial	1.64±0.04	0.63±0.02
Peritoneal	1.34±0.05	0.49±0.02
CSF	0.57±0.22	0.39±0.01

CRP estimation was done from various fluids of subjects in group B and group C and it was found that there was significant difference between the CRP levels of body fluids in group A and group C subjects which was also found to be statistically significant.

DISCUSSION

In present study, body fluids in some selected tuberculous cases and non-tuberculous cases for biochemical parameters such as ADA and CRP in some selected cases were also studied. The types of fluid examined are pleural, pericardial, peritoneal and CSF and was compared with the non-tuberculous cases. The fluids were analyzed for all these parameters

Atalay et al. (2006) [13] carried out study on pleural fluid ADA to differentiate between exudates and transudate. They claimed that pleural ADA separates pleural exudates from transudates. In addition , a cut-off point of 15.3 IU/L for pleural ADA test yielded as sensitivity and specificity of 85.8% and 82.3% and at a cut-off point of 0.66 for pleural fluid/serum ADA ratio yielded 83.3% and 83.2% respectively in their study which is comparable to present study's cut-off point of 15 I U/L which yielded

the sensitivity and specificity of pleural ADA concentration for the diagnosis of exudates to be 98% and 100% respectively and at cut-off point of 1.0 for pleural fluid/serum ADA concentration 100% and 96% respectively. Hence, the cut-off point established in this study yielded a better sensitivity and specificity as compared to the other study **(Jadhav and Bardapurkar 2007)** [14]. One study also showed that fluid cytology is more useful in diagnosis of malignant effusion compared with biopsy **(Prakash and Reiman 1985)** [15].

The present study showed that, ADA activity is increased in the body fluid of 89% of patients with tuberculous effusion with levels ranging from 20 to 30U/L. Similar results have been reported by other workers **(Paliwal 1998, Alatas 2000)** [16] but with some higher magnitude. High ADA levels have been reported in patients with empyema **(Jereb HGJ 2005)**[17] and parapneumonic effusion. **(Zink et al 2003)**[18] The ADA levels in malignant effusion are generally low

Rao et al (2009) [19] has found in their study that CRP levels were found to be significantly higher in sputum-positive group as compared with the

sputum-negative group. Statistically, the difference was found to be significant. **Luetkemeyer FA et al (2007)** [20] found during their study that the CRP levels decrease as the lesion become inactive or as the severity of the disease decreases. They included 157 patients in their study and concluded that the CRP levels decreases as the tubercular severity decreases.

Chieraku et al (2004) [21] investigated the diagnostic value of CRP in 72 patients for pleural effusion including 43 of tuberculous variety. They found that the mean serum CRP level was significantly higher than that compared with other patients

Baba et al (2008) [22] evaluated the immune responses in HIV infected patients with pleural TB by the OFT TB-G IFN- γ assay. They concluded that QFT-TB test in blood could contribute to the diagnosis of TB pleuritis in the HIV positive population. **Kim et al (2000)** [23] in their study evaluated the clinical significance of CRP, and ADA determinations in sera and pleural fluids from 37 patients with pleural effusion. CRP levels and ADA activities were found to be higher in exudates than in transudates of pleural fluids. They also reported significant a positive correlation between serum CRP levels and serum ADA values.

CONCLUSION:

In conclusion, the combined estimation of parameters such as ADA, CRP, and IFN- in serum as well as body fluids is of great value in the diagnosis and prognosis of these lethal tuberculous infections. This is because by combining all these parameters, i.e., ADA, CRP, and IFN-, the diagnosis of tuberculous infection becomes more easy, accurate, and quick. This emphasises the need for their continued use and to differentiate pulmonary and extra pulmonary tuberculosis.

The suggested cut-off value for the study's criteria produces a diagnosis of tubercular infection with excellent sensitivity and specificity. As a result, the current work suggests that additional research is needed to assess the value of newer parameters like IFN-v in the diagnosis of TB and its relationship with other serum markers and body fluid markers like ADA& CRP in order to enhance their diagnostic importance and improve the outcome of TB diagnosis.

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